Novel Application of MEMRI Detects Sensory Deficits from Olfactory System Injury in Newborn Rabbit after Antenatal Hypoxia-Ischemia

A. Drobyshevsky[1], A. M. Robinson[2], M. Derrick[1], A. M. Wyrwicz[3], X. Ji[1], I. Englof[1], S. Tan[1]

[1]Pediatrics, Evanston Northwestern Healthcare, Evanston, IL, United States, [2]Otolaryngology-Head and Neck Surgery, Northwestern University, Chicago, IL, United States, [3]Center for Basic MR Research, Evanston Northwestern Healthcare, Evanston, IL, United States

Introduction

Sensory deficits are frequently observed in cerebral palsy patients but there are very few studies of the sensory system that investigate hypoxia-induced injury. The newborn rabbit is born blind and deaf but has the ability to smell. Smell deficits elicited by the motor response are observed in our cerebral palsy model of antenatal hypoxia-ischemia in rabbits (Derrick M, et al 2004).

Hypothesis

Long lasting and selective olfactory tract injury occurs following fetal hypoxia

Methods

We modified previously developed neuronal tracing technique in olfactory system (Pautler and Koretsky, 2002) in order to quantitatively asses the difference in Mn^{2+} uptake and transport by olfactory epithelium neurons between control and post-hypoxic rabbit kits and whether this difference is augmented by specific odors.

Animal model. In vivo global hypoxia–ischemia of fetuses was induced by uterine ischemia in timed pregnant New Zealand white rabbits at 70% (E22) gestation as described previously (Derrick et al., 2004). Dams were allowed to deliver and postnatal day 1 (P1) kits underwent neurobehavioral testing, including the test for aversive reaction to amyl acetate, peppermint and ethanol.

Contrast delivery and odor stimulation . Rabbit kits (n=28) at postnatal age P1 were administered 20 mg/kg $MnCl_2$ in both nostrils. One of the nostrils was randomly plugged. A subset of animals (9 controls and 14 hypoxic) was given odor stimulation by blowing air passing through vaporizer with amyl acetate or clear air intermittently for 6 hours. Animals were also given the same stimulation, but without the contrast administration. All measurements were done at the same age P1,and same time - 6 hours after the contrast administration.

MRI imaging. Animals were sedated and imaged in 4.7T Bruker BioSpec scanner with a 30 mm birdcage coil. T2- (TR/TE=220/6 ms), T1- (TR/TE=4000/50 ms) - weighted images were acquired in the same slice position, 2 mm slice thickness, FOV 20 mm, and 128x64 matrix. The first and the last slices covered nasal turbinates with olfactory epithelium and portion of piriform cortex. To ensure reproducible imaging sections, 5 coronal slices, 2 mm thick and 0.5 mm inter-slice gap, were placed perpendicular to the plane connecting the most inferior point of cerebrum, as determined with the aid of multi-slice sagittal localizer scan, and the center of the 3rd slice was situated in the middle cross-section of olfactory bulbs. Region of interests (ROI) were placed on T2 image covering mid-septal portion of olfactory epithelium, glomerular layer of olfactory bulbs and medial prefrontal cortex; intensities from T1images were measured on selected ROIs. To account for the individual difference in brain tissue T1 time and amplifier gains, intensities derived from T1 image were normalized by values from the medial prefrontal cortex ROI, where there was no enhancement by Mn^{2+}

Histopathology. Kittens' heads were fixed in 4% paraformaldehyde, 10 µm paraffin sections of nasal turbinates and olfactory bulbs were stained for mature olfactory neurons using OMP antibody. Total count and number of mature neurons, olfactory epithelium thickness were measured in the nasal midseptum.

Results

Enhancement of olfactory epithelium and olfactory bulbs after nasal administration of $MnCl_2$ occurred in both closed and open nostrils (Fig. 1). Amyl acetate exposure resulted in increased augmentation of the signal on the open side in control group. There was a significant decrease in Mn^{2+} uptake in olfactory epithelium and olfactory bulbs on the open side in P1 kits following fetal hypoxia (Fig. 2). There was variability of the signal from the plugged side with some kits having enhancement even without odor (Fig.1 right bulb). Histopathology sections demonstrate significant decrease in olfactory epithelium thickness and number of mature olfactory neurons in hypoxic group.

Conclusions

1) Functional MRI studies are superior to neurobehavioral smell testing in the rabbit kits as they are more sensitive, quantifiable measures and do not depend upon the motor response.

2) Antenatal hypoxia-ischemia causes long lasting injury to neuronal tracts of the olfactory system including olfactory epithelium.

3) The difference between open and plugged nostrils may serve as an indicator of the potential for neuronal uptake and the effect of ambient odors on baseline function.

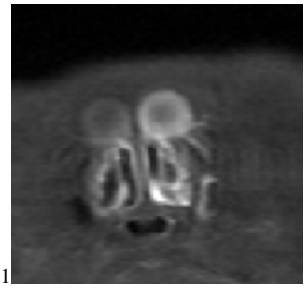

Fig.1

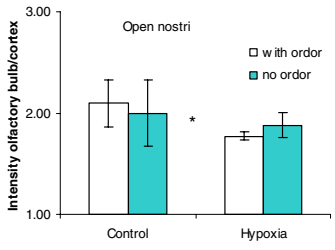

Fig. 2

Acknowledgements: NIH NS 43285,1 S10 RR15685

References

Derrick M, et al (2004) Preterm fetal hypoxia-ischemia causes hypertonia and motor deficits in the neonatal rabbit: a model for human cerebral palsy? J Neurosci 24:24-34.

Pautler, R.G., Koretsky A.P., 2002. Tracing odor-induced activation in the olfactory bulbs of mice using manganese-enhanced magnetic resonance imaging. Neuroimage 16(2):441-8.